

Amendments to the Drawings:

The attached sheets of drawings includes the addition of Figures 19-24.

REMARKS

As will be discussed in further detail below, the specification has been amended to correct the priority claim, to insert SEQ ID NOs, to correct editorial errors that have heretofore gone unnoticed and to remove drawings from the text of the specification. These drawings are now separate figures. The abstract has been amended as well.

Claims 91-103 are pending in the above-referenced application. Claims 91, 94, 99 and 103 have been amended to more distinctly claim that which Applicants regard as their invention. Specifically, claims 91 and 99 have been amended to particularly recite that a primer binding site is regenerated in said specific nucleic acid provided.

1. Priority

It is asserted that a proper priority claim was not made in the instant application. In response, Applicants assert that a proper priority claim was indeed made. A copy of Applicants transmittal letter is attached hereto for Examiner's reference. However, there was an improper designation of the filing date and serial number of the parent application of application serial number 10/260,031. The specification has been amended accordingly. Furthermore, Applicants herewith submit a petition to claim benefit under 35 USC 120, 121, or 365(c) of a prior copending nonprovisional application or international application designating the united states of america (37 C.F.R. 1.78(a)).

2. Abstract

The abstract of the disclosure is objected to because it exceed 150 words and uses the phrase "disclose in this invention". In response, Applicants herewith submit a revised abstract. Therefore, the objection has been overcome.

3. Specification

It is asserted that on pages 6, 8, 10, 12, 14 and 17, the specification contains a drawing. Applicants note that there are no drawings on these recited pages. However, Applicants did find drawings on pages 25, 30, 31, 32 and 43 and have amended these pages accordingly. The drawing on page 25 is now FIG. 19, the drawing on page 30 is

now FIG. 20, the drawings on page 31 are now FIGS. 21 and 22, the drawing on page 32 is now FIG. 23 and the drawing on page 43 is now FIG. 24.

4. Claim Objections

Claims 94 and 102 are objected to. It is asserted that DNA:RNA should be written as DNA:RNA. In response, claims 94 and 102 have been amended accordingly. Therefore, Applicants respectfully request that the objections be withdrawn.

5. Claim Rejections-35 USC 102

Claims 91-95 and 98 were rejected over Schuster et al. and claims 91-98 were rejected over Kacian et al. These are described in detail below.

5.1 Schuster et al.

Claims 91-95 and 98 are rejected under 35 U.S.C. 102(b) as being anticipated by Schuster et al. (US Patent 5,169,766 December 8, 1992). The Office Action specifically states

Schuster et al. teaches a method of amplifying a nucleic acid molecule. With regard to Claim 91, Schuster et al. teaches providing a DNA target and mixing the target with nucleoside triphosphates (Figure 1 Column 7, lines 60-65). Schuster et al. teaches the use of primers which are chemically modified by blocking the 3' terminus using a 3' terminal nucleotide lacking a 3' hydroxyl group (Column 11 lines 60-64). Schuster et al. teaches conditions or agents (nucleic acid producing catalysts) which increase amplification are present (Column 7, lines 50-55). Schuster et al. teaches that the assay mixture has a sufficient quantity of cofactors to support the degree of amplification desired (Column 7, lines 60-65). Schuster et al. teaches isostatic conditions, such as, the use of Tris base (pH stabilizer) in the amplification reactions, stable temperature of 37°C for 3 hours, and with a specific number of molecules of RNA (Column 13, lines 40-52). With regard to Claims 92 and 93, Schuster et al. teaches the use of RNase H to remove RNA from the cDNA (Column 8, lines 7-10). Schuster et al. teaches an mRNA promoter (primer) which is used to extend and make ssRNA (Figure 3). Schuster et al. teaches another primer (DNA) is annealed to the ssRNA and cDNA

is copied (Figure 3). Schuster et al. teaches the ssRNA (which is the extended promoter) is destroyed by RNase H. Further, any primers which are in the solution but did not primer to the original ssDNA would be destroyed by RNase H, therefore allowing for a reaction solution with only the cDNA that allows the completion of another cycle and the production of another cDNA strand identical to the ssDNA template. With regard to Claim 94, Schuster et al. teaches the primers can be DNA or RNA (Column 5 lines 35-38 and 55-60). With regard to Claim 95, Schuster et al. teaches the use of primers which are chemically modified by blocking the 3' terminus using a 3' terminal nucleotide lacking a 3' hydroxyl group (Column 11 lines 60-64). With regard to Claim 98, Schuster et al. teaches a promoter (primer) in which at least 1 nucleotide is noncomplementary (Fig 2 5th step).

Applicants respectfully traverse the rejection. Schuster does not contain each and every element of the claimed invention, the subject matter recited in claim 91.

Specifically the method of the present invention is directed to a method of producing one or more copies of a specific nucleic acid, wherein the products produced are substantially free of any primer sequences. The method of the invention comprises the following steps:

- (a) providing a sample containing or suspected of containing the sequence of said specific nucleic acid;
- (b) contacting said sample with a mixture comprising:
 - (i) unmodified nucleic acid precursors,
 - (ii) one or more specific chemically-modified primers each of which primer is substantially complementary to a distinct sequence of said specific nucleic acid, and
 - (iii) an effective amount of a nucleic acid producing catalyst;
- (c) allowing the mixture of step (b) to react under isostatic conditions of temperature, buffer and ionic strength to produce at least one copy of said specific nucleic acid; and
- (d) removing all primer sequences from the product produced in step (c), the copy of the specific nucleic acid, to regenerate a primer binding site **on said specific nucleic acid**. As a result, a new priming event occurs and produces more than one copy

of said specific nucleic acid.

It is Applicants view that Schuster does not teach step (d). Specifically, in Applicants method, only the primer portion of the extended primer generated in step (c) is actually removed from the specific nucleic acid so that a binding site is regenerated on said specific nucleic acid. As a result, more primer binding and extension events from the same specific nucleic acid occurs. In contrast, in Schuster, RNA copies are made off of the original template. Primer binding events only take place on resultant copies. In Schuster, the primer is not removed from the original template. Therefore, it would not be possible to regenerate a primer binding site on the specific nucleic acid using the method of Schuster.

Applicants note that claims 92-95 and 98 depend from claim 91. Therefore, arguments made with respect to claim 91 would be applicable to claims 92-95 and 98 as well.

In view of the above argument, Applicants assert that the rejections of claims 91-95 and 98 have been overcome. Therefore, Applicants respectfully request that the rejection be withdrawn.

5.2 Kacian

Claims 91-98 are rejected under 35 U.S.C. 102(b)/102(e) as being anticipated by Kacian et al. (US Patent 555451 6 September 10, 1996). The Office Action specifically states

Kacian et al. teaches a method of amplifying a target nucleic acid sequence (Abstract). With regard to Claim 91, Kacian et al. teaches a method of incubating a promoter-primer and a target sequence in DNA priming and nucleic acid synthesizing conditions (ribonucleotide triphosphates and deoxyribonucleotide triphosphates) for a period of time to many multiple copies of the target sequence (Column 10 lines 23-33). Kacian et al. teaches using a DNA polymerase (nucleic acid producing catalyst) (Column 10 line 59). Kacian et al. teaches that the reaction takes place under conditions that are substantially isothermal and include substantially constant ionic strength and pH, i.e. isostatic conditions (Column 10 lines 37-45). With regard to Claims 92-93, Kacian et al. teaches that generation of target sequence is done using RNase H (Column 4 lines 65-67

and Column 5 lines 1-5). Kacian et al. teaches the promoter-primer may be altered with ribonucleotides (Column 9, line 15). Therefore Kacian et al. teaches a reaction in which RNase H is in the presence of a RNA-DNA hybrid (DNA target with a promoter with ribonucleotides), it is inherent that the RNase H will denature the ribonucleotide promoter and thereby release the DNA target from the promoter. With regard to Claim 94, Kacian et al. teaches the use of DNA as a primer sequence (Column 6 lines 18-25). Kacian et al. teaches that this sequence may have modifications such as dideoxynucleotide residues that have been modified such as phosphorothioates (chemically) (Column 9, lines 14-16). With regard to Claim 95, Kacian et al. teaches the 3' end of the promoter-primer may be modified (Column 7, line 6). With regard to Claim 96, Kacian et al. teaches that one modification can be the addition of a phosphorothioate (sulphur heteroatom) (Column 9 lines 17). With regard to Claim 97, Kacian et al. teaches that promoter-primer can include the addition of 3'2' dideoxynucleotide residues modified with phosphorothioates (Column 9 lines 15-17). With regard to Claim 98, Kacian et al. teaches a promoter primer which has at least one nucleotide that is noncomplementary (Figure 1).

Applicants respectfully traverse the rejection. As with Schuster, Kacian et al. does not teach each and every element of the method of the present invention, the method recited in claim 91. It is Applicants view that Kacian et al. like Schuster does not teach step (d). As noted above, in Applicants method, only the primer portion of the extended primer generated in step (c) is actually removed from the specific nucleic acid so that a binding site is regenerated on said specific nucleic acid thereby allowing more primer binding and extension events from the same specific nucleic acid. In contrast, in Kacian et al., RNA copies are made off of the original template. Primer binding events only take place on resultant copies. In Kacian et al., the primer is not removed from the original template. Therefore, it would not be possible to regenerate a primer binding site on the specific nucleic acid using the method of Kacian et al.

Claims 92-98 depend from claim 91. Therefore, arguments made with respect to claim 91 would be applicable with respect to claim 91.

In view of the above arguments, Applicants assert that the rejection of claims 91-98 under 35 USC 102 over Kacian has been overcome. Therefore, Applicants

respectfully request that the rejection be withdrawn.

6. The Rejections under 35 USC § 103

The rejections under 35 USC 103 are set forth below.

6.1 The Rejections over Schuster in view of Skerra

Claims 96-97 are rejected under 35 U.S.C. 103(a) as being unpatentable over Schuster et al. (US Patent 5,169,766 December 8, 1992) in view of Skerra (Nucleic Acids Research 1992 Vol. 20 p. 3551). The Office Action specifically states:

Schuster et al. teaches a method of amplifying a nucleic acid molecule. Schuster et al. teaches providing a DNA target and mixing the target with nucleoside triphosphates (Figure 1 Column 7, lines 60-65). Schuster et al. teaches the use of primers which are chemically modified by blocking the 3' terminus using a 3' terminal nucleotide lacking a 3' hydroxyl group (Column 11 lines 60-64). Schuster et al. teaches conditions or agents which increase amplification are present (Column 7, lines 50-55). Schuster et al. teaches that the assay mixture has a sufficient quantity of cofactors to support the degree of amplification desired (Column 7, lines 60-65). Schuster et al. teaches the use of RNase H to remove RNA from the cDNA (Column 8, lines 7-10).

Schuster et al., however, does not teach primers modified by heteroatoms comprised of nitrogen or sulfur and chemically modified primers comprised of nucleoside triphosphates.

Skerra teaches a method of using phosphorothioate primers in an amplification method (Abstract). With regard to Claims 96-97, Skerra teaches the modification of primers by the addition of a single phosphorothioate bond (heteroatom of sulfur) at the first 3' terminal internucleotide linkage during synthesis of the oligodeoxynucleotide (p.3552 1st column last paragraph). Skerra teaches that the phosphorothioate bond is much less favored substrate to nuclease activity than the naturally occurring phosphodiester bond (P. 3552 1st column last sentence and 2nd column 1st sentence).

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Schuster et al., to use the

phosphorothioate primers as taught by Skerra. The ordinary artisan would have been motivated to modify the method of Schuster et al. because Skerra teaches the use of phosphorothioate primers would avoid the lower PCR yield and non-specific side products resulting from 3' terminal editing of the primer molecule by protecting the oligodeoxynucleotide from a 3' terminal exonucleolytic attack (p. 3553 2nd column last paragraph).

Applicants respectfully traverse the rejection. As argued above, Schuster et al. does not disclose the method recited in amended claim 91. Claims 96-97 depend from claim 91. It would not be obvious to combine the disclosures of Schuster et al. with Skerra. Schuster et al. is directed to amplification and Skerra is directed to oligonucleotide analogs. There was no suggestion regarding the use of analogs in the amplification method of Schuster. Furthermore, it is admitted in the Office Action that Schuster et al. does not disclose chemically modified primers.

Furthermore, as noted above, there is no disclosure of step (d) recited in claim 91 in Schuster et al. Therefore, even if the disclosures of Schuster et al. and Skerra are combined, the method of the present invention would not be obtained given the lack of disclosure of step (d). Even assuming *arguendo* that it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Schuster et al., to use the phosphorothioate primers as taught by Skerra and/or the ordinary artisan would have been motivated to modify the method of Schuster et al., the claimed method would have not been obtained since step (d) is not disclosed.

In view of the above arguments, Applicants assert that the rejection of claims 96-97 under 35 USC 103 have been overcome. Therefore, Applicants respectfully request that the rejections be withdrawn.

6.2 The Rejections over Schuster et al. in view of Cerretti

Claims 99-103 are rejected under 35 U.S.C. 103(a) as being unpatentable over Schuster et al. (US Patent 5,169,766, December 8, 1992) in view of Cerretti et al. (US Patent 5,317,087, May 31, 1994). It is specifically stated

Schuster et al. teaches a method of amplifying a nucleic acid molecule. With regard to Claim 99, Schuster et al. teaches providing a DNA target and mixing the target with

nucleoside triphosphates (Figure 1 Column 7, lines 60-65). Schuster et al. teaches conditions or agents which increase amplification are present (Column 7, lines 50-55). Schuster et al. teaches that the assay mixture has a sufficient quantity of cofactors to support the degree of amplification desired (Column 7, lines 60-65). With regard to Claims 100 and 101, Schuster et al. teaches the use of RNase H to remove RNA from the cDNA (Column 8, lines 7-10). With regard to Claim 102, Schuster et al. teaches the primers can be DNA or RNA (Column 5 lines 35-38 and 55-60). With regard to Claim 103, Schuster et al. teaches a promoter (primer) in which at least 1 nucleotide is noncomplementary (Fig 2 5th step).

Schuster et al., however, does not teach primer hybridization in which at least one loop structure is formed.

Cerretti et al. teaches that a library of cDNA can be prepared by using hairpin loop primers (Column 11 lines 10-26). Cerretti et al. teaches the mRNA primer is hybridized to a first cDNA strand (Column 11 lines 10-26). Cerretti et al. teaches that this results in a "hairpin" loop at the 3' end of the initial cDNA strand that serves as an integral primer for the second DNA strand (Column 11 lines 10-26). Cerretti et al. teaches that the second cDNA strand is synthesized using a DNA polymerase and the hairpin loop is cleaved to produce double stranded cDNA molecules (Column 11 lines 10-26).

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Schuster et al. to use the hairpin loop primer as taught by Cerretti et al. The ordinary artisan would have been motivated to modify the method of Schuster et al. because Cerretti et al. teaches a method of using hairpin loops to copy small cDNA fractions from a large cDNA template (Column 11 lines 10-26). The ordinary artisan would want to use hairpin loops as a way to prepare a library of double-stranded cDNA and would want to cleave the mRNA primer from the target cDNA in order to keep using the original long strand of cDNA.

The ordinary artisan would therefore be able to produce multiple copies at multiple positions of the target cDNA strand by annealing a mRNA primer, copying a fragment of cDNA with a hairpin loop, removing the mRNA primer, and adding another mRNA primer somewhere else on the target DNA.

Applicants respectfully traverse the rejection. First, it is Applicants view that it would not be obvious to modify the method of Schuster et al. to use the hairpin loop primer as taught by Cerretti et al. or alternatively to modify the method of Schuster et al. As noted above, in the method disclosed by Schuster et al., there is no disclosure of step (d), regeneration of the primer binding site on said specific nucleic acid.

Furthermore, the use of the hairpin loop in Cerretti can be distinguished from its use in the method of the present invention. This is because, in Cerretti, it is the stem portion that is acting as the primer. Therefore, again, there can be no regeneration of the primer binding site. In contrast, in the method of the present invention, the hairpin portion acts as the primer.

Even assuming *arguendo* that it would have been obvious to combine the disclosures of Schuster et al. with Cerretti et al., or modify the method of Cerretti et al. with Cerretti et al., the claimed method would have not been obtained. In Cerretti, the stem portion of the hairpin loop is functioning as a primer by carrying out self hybridization followed by extension. When the nuclease is used for digestion, the segment that was formerly the stem portion remains intact and bound to the template and it is only the loop portion that is digested. As such, the segment of the template that was used as a "primer binding site" remains double-stranded and another primer binding event doesn't take place as required by claim 99.

In view of the above arguments, Applicants assert that the rejection of claims 96-97 under 35 USC 103 have been overcome. Therefore, Applicants respectfully request that the rejections be withdrawn.

6.3 The Rejections of Kacian et al. in view of Cerretti

Claims 99-103 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kacian et al. (US Patent 5554516 September 10, 1996) in view of Cerretti et al. (US Patent 5,317,087 May 31, 1994). The Office Action specifically states:

Kacian et al. teaches a method of amplifying a target nucleic acid sequence (Abstract). With regard to claim 99, Kacian et al. teaches a method of incubating a promoter-primer and a target sequence in DNA priming and nucleic acid synthesizing conditions (ribonucleotide triphosphates and deoxyribonucleotide triphosphates) for a period of time

to many multiple copies of the target sequence (Column 10 lines 23-33). Kacian et al, teaches using a DNA polymerase (nucleic acid producing catalyst (Column 10 line 59). Kacian et al. teaches that the reaction takes place under conditions that are substantially isothermal and include substantially constant ionic strength and pH (Column 10 lines 37-40). With regard to Claims 100-101, Kacian et al. teaches that generation of target sequence is done using RNase H (Column 4 lines 65-67 and Column 5 lines 1-5). With regard to Claim 102, Kacian et al. teaches the use of DNA as a primer sequence (Column 6 lines 18-25). With regard to Claim 103, Kacian et al. teaches a promoter primer which has at least one nucleotide that is noncomplementary (Figure 1).

Kacian et al., however, does not teach primer hybridization in which at least one loop structure is formed.

Cerretti et al. teaches that a library of cDNA can be prepared by using hairpin loop primers (Column 11 lines 10-26). Cerretti et al. teaches the mRNA primer is hybridized to a first cDNA strand (Column 11 lines 10-26). Cerretti et al. teaches that this results in a "hairpin" loop at the 3' end of the initial cDNA strand that serves as an integral primer for the second DNA strand (Column 11 lines 10-26). Cerretti et al. teaches that the second cDNA strand is synthesized using a DNA polymerase and the hairpin loop is cleaved to produce double stranded cDNA molecules (Column 11 lines 10-26).

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Kacian et al. to use the hairpin loop primer as taught by Cerretti et al. The ordinary artisan would have been motivated to modify the method of Kacian et al. because Cerretti et al. teaches a method of using hairpin loops to copy small cDNA fractions from a large cDNA template Column 11 lines 10-26). The ordinary artisan would want to use hairpin loops as a way to prepare a library of double-stranded cDNA and would want to cleave the mRNA primer from the target cDNA in order to keep using the original long strand of cDNA. The ordinary artisan would therefore be able to produce multiple copies at multiple positions of the target cDNA strand by annealing a mRNA primer, copying a fragment of cDNA with a hairpin loop, removing the mRNA primer, and adding another mRNA primer somewhere else on the target DNA,

Applicants respectfully traverse the rejection. As argued above with respect to claim 91, Kacian et al. does not disclose step (d) of claim 99, regeneration of the primer binding site of the specific nucleic acid. Similarly, given that it is the stem in the hairpin loop of Cerretti that acts as a primer, as discussed above, there could be no regeneration of the primer binding site of the specific nucleic acid using the Cerretti primer. Therefore, even assuming *arguendo* that it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Schuster et al., to use the hairpin structures as taught by Cerretti and/or the ordinary artisan would have been motivated to modify the method of Kacian et al., the claimed method would have not been obtained, since step (d) could not be accomplished given the disclosures of both of these references.

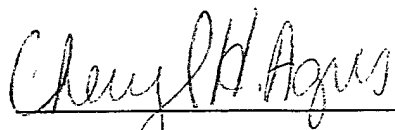
In view of the above arguments, Applicants assert that the rejection of claims 96-97 under 35 USC 103 have been overcome. Therefore, Applicants respectfully request that the rejections be withdrawn.

7. Conclusions

In view of the foregoing, Applicants assert that the claims are now in condition for allowance. Early action to that end is respectfully requested. The Examiner is invited to contact the undersigned at (914) 712-0093 if she has any questions.

Respectfully submitted,

Date: 11/17/06


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